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Development and Testing of a Laboratory Spray Table Methodology to Bioassay Simulated Levels of Aerial Spray Drift

ABSTRACT: The objective of this work was to develop a repeatable methodology for bioassaying simulated levels of aerially applied glyphosate at deposition levels ranging from 1/3 to 1/100 of labeled rate at droplet sizes of 100 μm in a spray table environment. These drift deposition levels are consistent with downwind drift measurements out to 200 m seen in previous field studies focusing on quantitative drift assessment. Additionally, full rate applications were included for comparative purposes. The deposition levels were obtained by varying nozzle traverse speed and plant location under the nozzle. Ten replications were conducted at each targeted rate applying glyphosate to container grown-plant samples. Deposition was measured on Mylar cards through fluorometric analysis. Plant health measures [height and normalized difference vegetation index (NDVI)] were taken at 0, 1, 3, 5, 7, and 14 days after treatment. An equal number of nontreated control plants were analyzed alongside treated plants. Deposition and plant health data were used to generate dose-response relationships. Dose-response curves relating change in plant height and change in measured NDVI values corresponding to deposition levels were generated. This methodology is one that can be implemented across a wide variety of plant and pesticide combinations. Collected data from this and future studies will be tested under field conditions and ultimately be included in application decision support systems that integrate spray drift modeling results with established dose-response relationships.

KEYWORDS: spray drift, bioassay, dose-response, spray drift simulation

Introduction

Spray drift has always been one of the major concerns in the application industry. Spray drift is defined by the U.S. Environmental Protection Agency (EPA) as "…the physical movement of pesticide droplets or particles through the air at the time of pesticide application or soon thereafter from the target site to any non- or off-target site" [1]. Spray drift research typically focuses on the amount and the consequences of spray drift or application technologies and methodologies to minimize drift. There is a large body of literature, spanning several decades, detailing the degree of spray drift resulting from agricultural applications as a result of meteorological conditions [2–5], equipment type and operational parameters [6–8], crop type [9,10], and spray material [11]. The Spray Drift Task Force compiled a database of reported spray drift data [12] which supported the further development and evaluation of the spray drift model AgDRIFT [12,13]. All of these studies provide a solid foundation detailing principal causes of spray drift and the magnitude and characteristics of the drifting material. None of these studies addressed the biological effects resulting from the drifting material.

The few studies that dealt with biological effects used handheld or ground-based spray systems to apply a spray product at levels simulating those that resulted from spray drift. These studies contained a variety of crops that include, but were not limited to, wheat [14], sugarcane [15], alfalfa [16], soybeans [17], maize [18], native plants [19], insects such as bees [20] and butterflies [21], and aquatic ditches [22]. Deposition rates reported in these studies varied from 0.1 to 50 % of labeled product application rate, and biological assessments ranged from visual damage to yield assessments for plants and mortality for insects. Varied results from these studies support previous findings that the effect from a given product at a given dosage is product and species dependent [12]. Two notable aerial application studies integrated biological samples into the downwind sampling scheme. Ray et al. [23] used tomato plants alongside fallout plates to determine biological effects of glyphosate spray drift out to 80 m resulting from a helicopter application.

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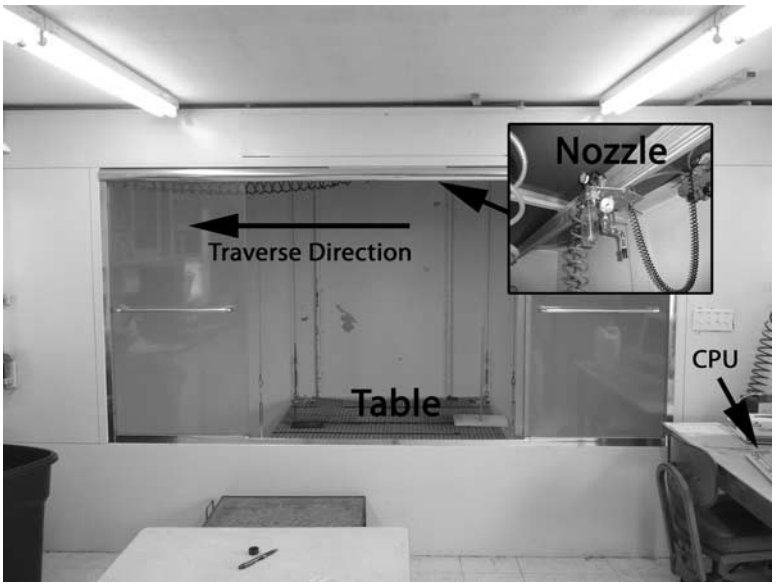

FIG. 1—*Spray table setup.*

Marrs et al. [24] used common sorrel alongside water-sensitive cards to measure the biological response to spray drift from a helicopter application out to 240 m. The major shortcoming of these studies was the lack of depositional characteristics reported; i.e., target coverage and droplet size data, as these data may be critical to the biological impacts seen off target. The objective of this work was to develop a repeatable methodology for bioassaying simulated levels of aerially applied glyphosate at deposition levels ranging from 1/3 to 1/100 of labeled rate at a 100-μm droplet size in a spray table environment.

Methods

Spray Treatment Setup

A laboratory spray table was used to expose greenhouse-grown bermudagrass (Mirage Bermudagrass; Ferry Morse Seed Co., Fulton, KY) samples to simulated spray drift levels. The spray table (4.7 by 2.3 by 1.2 m) was equipped with a nozzle traverse system that permitted the nozzle assembly to traverse the entire length of the spray chamber. An adjustable table approximately 1.4 m below the nozzle could be set to between 0.3 and 1.5 m below the nozzle (Fig. 1). A rodless pneumatic cylinder was used to move the spray nozzle assembly over the table. The nozzle assembly was plumbed and fed spray material from a pressured bottle. Spray pressure could be varied between 0 and 827 kPa (0 and 120 psi), and traverse speeds could be varied between 0.5 and 6.7 m/s (1 and 15 mph).

Prior to applications of active ingredient, nozzle traverse speeds and plant locations under the nozzle were established. Targeted fractional dosage rates ranged from 1/3 to 1/100 of a labeled application spray rate of 46.8 L/ha (5 gpa). These ranges are consistent with field-collected drift measurements out to 200 m (650 ft) [25]. The 46.8-L/ha (5-gpa) rate is representative of a typical aerial application herbicide spray rate. An active ingredient rate of 2.3 L/ha (1 qt/acre) was selected based on label recommendations for Buccaneer® (glyphosate 41 %; Tenkoz Inc., Alpharetta, GA). A hollow cone nozzle (0.4–80—white; Ecologic Technologies, Inc., Pasadena, MD) was selected to generate the spray for the fractional deposition levels. A full rate treatment was also applied using a flat fan nozzle (9502EVS; Spraying Systems Co., Wheaton, IL). A Sympatec Helos laser diffraction droplet sizing system (Sympatec Inc., Clausthal, Germany) was used to measure droplet size. The Helos system uses a 623-nm He-Ne laser and was fitted with an R5 lens, which resulted in a dynamic size range of 0.5 μm to 875 μm in 32 sizing bins. Tests were performed within the guidelines provided by ASTM Standard E1260 [26]. Droplet sizing data measured included volume median diameter (VMD), the 10 % and 90 % diameters ($D_{V0.1}$ and $D_{V0.9}$) as defined in ASTM Standard E1620 [27]. Additionally, the flow rates for each nozzle were determined by operating the nozzle at the specified spray pressure for 15 s, collecting the spray material in a graduated

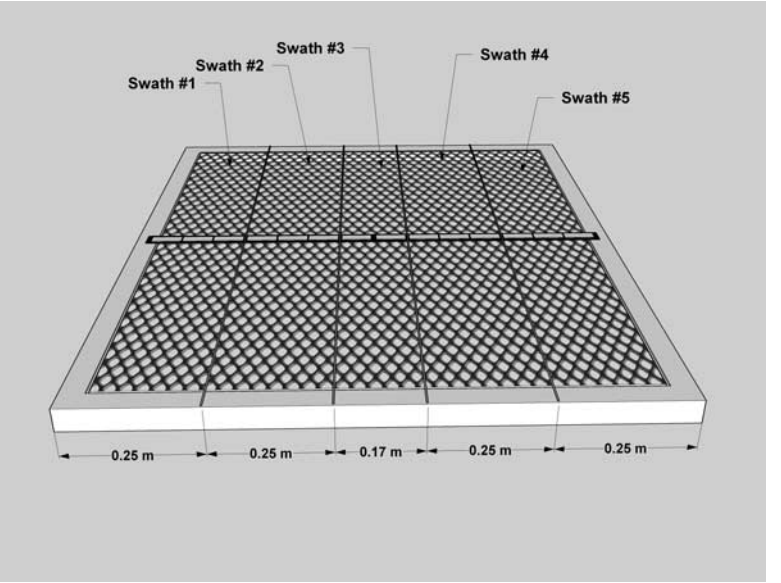

FIG. 2—*Spray table platform swaths.*

cylinder, then determining the volume collected. Three replicated measures were made for each nozzle/pressure combination.

For the hollow cone nozzle, the target VMD of 100 μm was observed at 241 kPa (35 psi). The resulting $D_{V0.1}$ and $D_{V0.9}$ values were 53 and 157, respectively. The nozzle flow rate was 14 mL/min. For the 9502EVS flat fan nozzle operating at 241 kPa (35 psi), the VMD was 243 μm, while the $D_{V0.1}$ and $D_{V0.9}$ were 97 and 373, respectively. The flow rate was 0.9 L/min.

A series of deposition trials were conducted over the range of nozzle traverse speeds [0.9–5.4 m/s (2–12 mph)] while measuring deposition at multiple positions under the nozzle. The hollow cone nozzle was tested; deposition for the flat fan nozzle was available from previous testing. The spray table platform was divided into five swaths (Fig. 2). For each speed evaluated, three Mylar cards were placed in Swaths 2 and 3. A spray pass was made over the table with a spray solution consisting of water, Caracid Brilliant Flavine FFN, a fluorometric tracer dye (0.264 g/L), and a nonionic surfactant (0.1 % v/v). Spray passes were replicated three times for spray nozzle traverse speeds of 0.5, 2.2, 4.5, 6.7, and 8.9 m/s (1, 5, 10, 15, and 20 mph). The sprayed Mylar cards were removed from the table after each replication and placed into individually labeled plastic bags. The bags were brought back to the laboratory for processing. After pipetting 40 mL of ethanol into each bag, the bags were agitated, and 6 mL of the effluent was poured into a cuvette. The cuvettes were then placed into a spectrofluorophotometer (Shimadzu, Model RF5000U, Kyoto, Japan) with an excitation wavelength of 423 nm and an emission at 489 nm. The fluorometric readings were converted to $\mu L/cm^2$ using a projected area of the sampler and by comparisons to standards generated using samples of spray solution. The minimum detection level for the dye and sampling technique was 0.07 ng/cm^2. The resulting deposition and speed data were used to generate a curve fit for each swath [Eqs 1 and 2]. The constants were derived by fitting a polynomial curve to the measured deposition and corresponding nozzle traverse speed data. Deposition values here and throughout the remainder of the manuscript are defined as fractional deposition values and are expressed as a percentage of the targeted full rate of 46.8 L/ha (5 gpa)

$$\text{Swath 2: Deposition} = 31.35\ (\text{speed})^{-0.65} \quad R^2 = 0.93 \tag{1}$$

$$\text{Swath 3: Deposition} = 22.67\ (\text{speed})^{-1.44} \quad R^2 = 0.53 \tag{2}$$

where:

deposition = percentage of 46.8 L/ha targeted full rate deposition and
speed = speed (m/s).

Based on Eqs 1 and 2, the treatments with the specified nozzle speeds and plant location were assigned (Table 1). Containers were numbered 1–70 with each group of ten being assigned to a treatment (Table 1).

TABLE 1—*Spray table treatments and operating parameters.*

Treatment	Plants	Targeted application rate (Fraction of 46.8 L/ha)	Nozzle	Pressure kPa (gpa)	Traverse speed m/s (mph)	Swath No.
1	1–10	1/3	0.4–80 Hollow cone	241 (35)	0.9 (2)	3
2	11–20	1/10	0.4–80 Hollow cone	241 (35)	5.4 (12)	3
3	21–30	1/33	0.4–80 Hollow cone	241 (35)	4.0 (9)	2
4	31–40	1/67	0.4–80 Hollow cone	241 (35)	6.3 (14)	2
5	41–50	1/100	0.4–80 Hollow cone	241 (35)	8.5 (19)	2
6	51–60	1	9502EVS Flat fan	241	6.3 (14)	3
7	61–70	0	none	···	···	···

Bioassay Studies

With the treatments established, bioassays were conducted on greenhouse-grown bermudagrass. Initially, 100 window boxes [45.7 cm by 19.1 cm (18 by 7.5 in.) Model DCB18 TC, Duraco Products Inc., Streamwood, IL] were seeded and placed in a greenhouse. The window boxes contained a purely organic seed starting mix into which the seeds were placed. The greenhouse vent fans were operated to allow air temperatures and humidities to follow outside ambient air conditions. Over the study the greenhouse temperature ranged from 18 to 24°C (65–75°F) at night and 24–32°C (75–90°F) during the daytime. After 2 weeks, only 70 containers which contained uniform healthy plants were included in the study. Each treatment was applied to ten containers. Prior to treatment, each container was scanned using a Greenseeker Hand Held Sensor (Model 505, NTech Industries, Inc., Ukiah, California) to measure the normalized difference vegetation index (NDVI) which is directly related to photosynthetic capacity [28]. The sensor was traversed over each container perpendicular to the long edge of the container for four passes (Fig. 3) at a traverse speed of 5 cm/s (2 in./s). The instrument head was held 76 cm (30 in.) over the top of the plant surface. NDVI readings were recorded at a rate of 10 Hz to a personal digital assistant (PDA). All scans for the study were conducted within a 60-min timeframe, thus ensuring minimal variation in ambient conditions. The recorded data were processed by averaging the maximum 40 NDVI values over all four passes. The plant height was measured using a ruler [measurements taken to the nearest 0.64 cm (0.25 in.)] and taking a visual reading at three locations (center of each third of the container) (Fig. 4).

After preapplication plant health measures were completed the spray table treatments were made. Spray solutions contained water, a fluorometric tracer dye (Caracid Brilliant Flavine FFN at 0.264 g/L), Buccaneer® (Tenkoz Inc., Alpharetta, GA; glyphosate 41 %; at 50 mL/L), and a nonionic surfactant (at 0.1 % v/v). For each plant treated, Mylar cards were placed at the leading and trailing edges of the container at the height of the soil layer for a measure of deposition (Fig. 5). The Mylar cards provided a consistent measure of active ingredient deposition (in terms of spray droplet impaction and collection characteristics) for use in dose-response determinations. After treatment, the cards were collected into

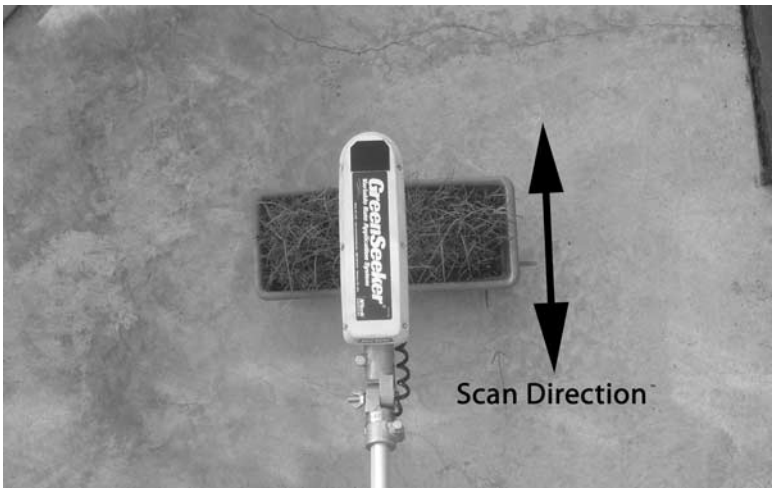

FIG. 3—*Greenseeker hand-held sensor scanning bermudagrass plant sample for NDVI.*

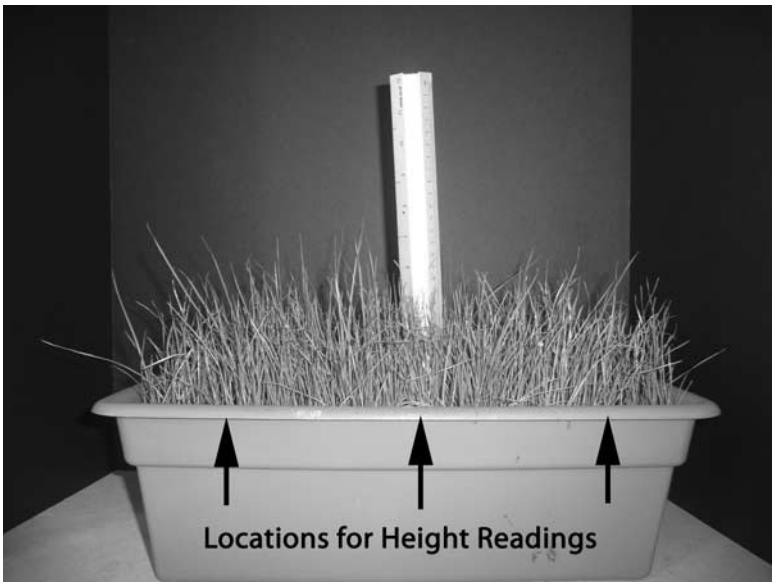

FIG. 4—*Measurement of plant height.*

labeled plastic bags and processed in the laboratory for deposition (volume/unit area). NDVI and plant height measurements were made following the pretreatment protocols at 2, 5, 7, and 9 days after treatment (DAT).

Data Analysis

Dose-response curves for the deposition and plant height and NDVI measures were fitted with CURVEEXPERT (Daniel Hyams, 1995–2001; Version 1.38) using the CurveFinder algorithm. All correlation analyses were performed using the PROC CORR procedures in SAS (SAS Inc., Cary, NC, Version 9.2). This procedure computed the Pearson's correlation coefficient between variables of interest. Only the fractional deposition rates were included in the curve fit and correlation analysis as they are representative of driftable materials. The full rate treatments consisted of larger droplet sizes corresponding to in-swath deposition and were included as an indication of full rate treatment effects. While data were measured at 2, 5, 7, and 9 DAT, only the 9 DAT data are included in the dose/response curve fit analysis. As the optimum DAT sampling time was not known, the additional days were included to ensure that the biological responses were adequately measured.

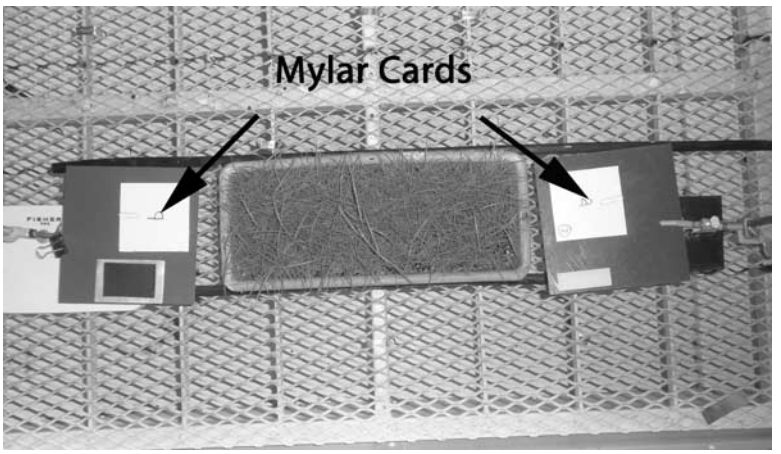

FIG. 5—*Placement of Mylar cards for deposition assessment during bioassay treatments.*

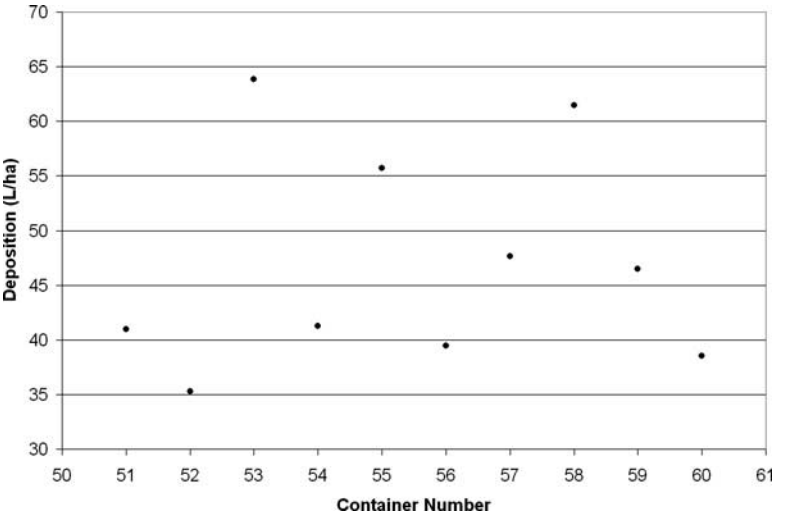

FIG. 6—*Measured deposition (L/ha) on containers treated at targeted deposition rate of 46.8 L/ha.*

Results

Deposition

Deposition on containers treated at the targeted full rate of 46.8 L/ha (5 gpa) (Containers 51–60) ranged from 35 to 64 L/ha (3.7 to 6.8 gpa) with an overall average of 47.0 L/ha (5 gpa) (Fig. 6). The actual fractional deposition levels ranged from 56 % to less than 1 % (Fig. 7). While the actual measured fractional deposition amounts were, in many cases, different from the targeted amount, overall the ranges of desired fractional deposition rates were well covered.

Plant Height

The initial average starting plant height across all 70 containers was 7.6 cm (3 in.). The measured data over the 9-day period were expressed as a percentage change in height from the initial state. Generally, containers treated with higher fractional rates showed less growth than those at lower rates. Containers treated with higher fractional rates (those above 1/10) were not much different than those treated at the full rate (−1 % change in height versus −3 % change), but were clearly different from the untreated containers (122 % change in height). Typical changes in plant height over the 9-day period after treatment varied with deposition rate. Typically, higher rates saw the maximum height either before treatment or 2 DAT, with

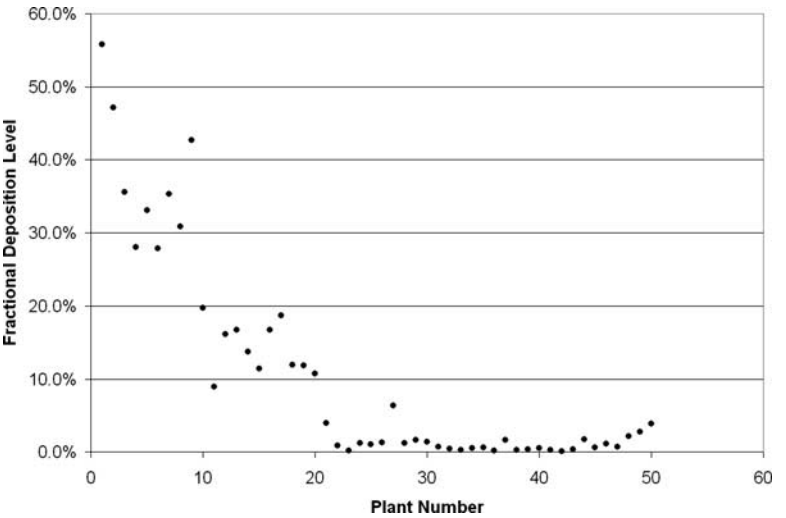

FIG. 7—*Fractional deposition levels by plant number for Treatments 1–5.*

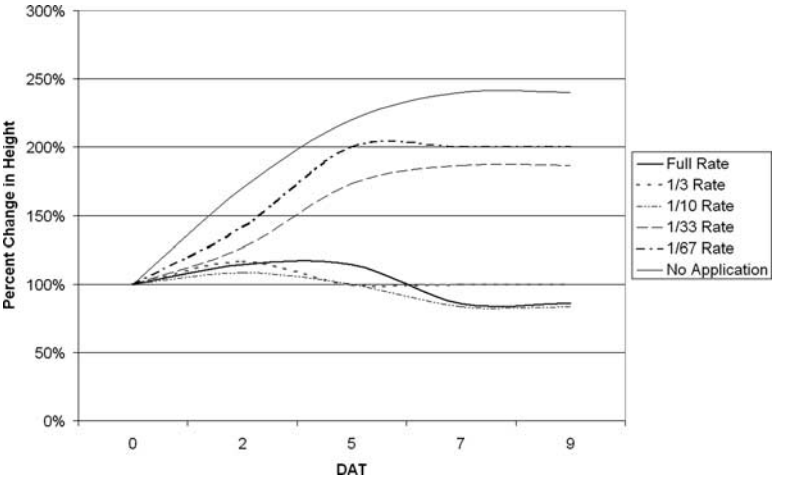

FIG. 8—*Typical changes in plant height at varying depositional rates over the days sampled.*

either no growth, or plants dying after that point. The lower rates saw plant continue to grow, eventually tapering off further growth after 5–7 DAT. These general trends can be seen in Fig. 8.

Correlation analysis shows a negative correlation between the fraction applied and the percent change in plant height (PCC=−0.67, $P<0.0001$, and $n=60$). The best fit model was a Logistic model [Eq 3 and Fig. 9] with a standard error of 35.4 and a correlation coefficient of 0.77. This relatively poor fit was deemed acceptable due the inherent variability of the measured plant height data. As these heights were measured by eye with a ruler and with natural variations in growth rate due to soil moisture conditions, this variability is not surprising. There was also high variability in untreated plants and plants treated at the lowest levels likely due to a gradient in the greenhouse temperature (6 °C) from one side of the growing table to the other that was discovered at the conclusion of the study. This meant that within treatment groups there was likely a 3–5 °C temperature difference which would have affected soil moisture and growth rates.

$$\text{Logistic model: } y = a/(1 + b^{*}e^{-cx}) \tag{3}$$

where:

x=deposition (percent of 46.8-L/ha rate),
y=percent change in height 9 DAT,
a=−221,
b=−3.2, and
c=−0.166.

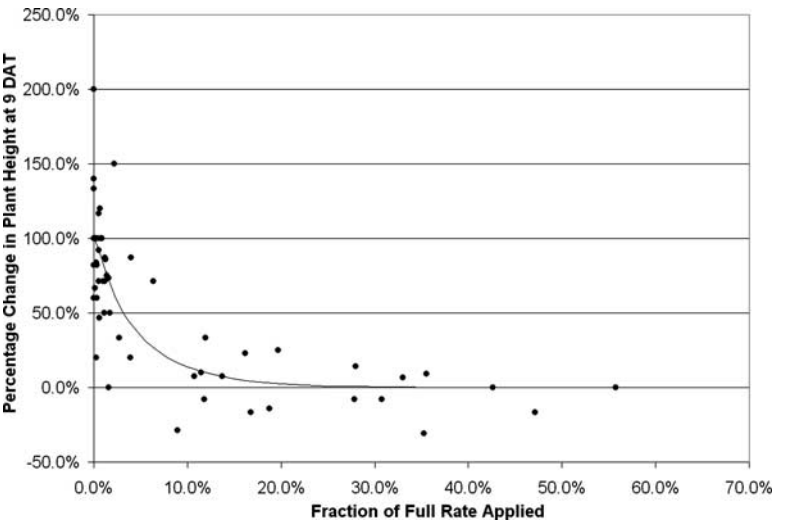

FIG. 9—*Plot and curve fit of deposition rate versus percent change in plant height.*

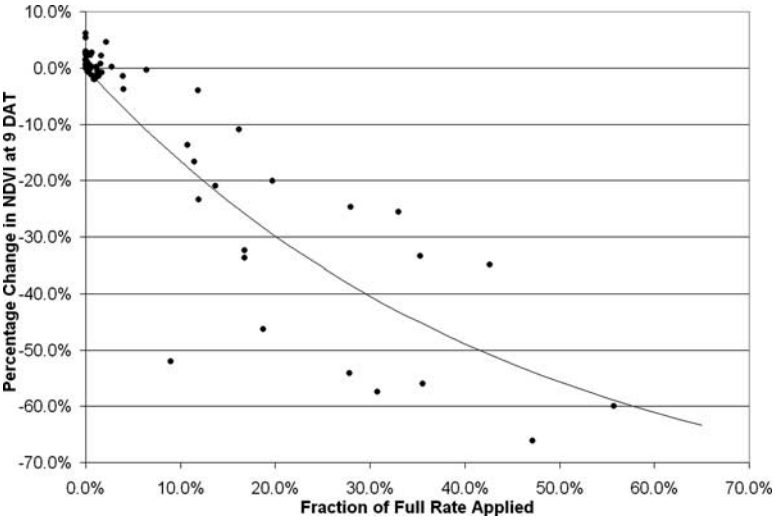

FIG. 10—*Plot and curve fit of deposition rate versus percent change in NDVI.*

NDVI

The initial average NDVI across all 70 containers was 0.95. The measured data over the 9-day period were expressed as a percentage change in NDVI from the initial state. Generally, containers treated at higher rates showed a greater reduction in NDVI than those treated at lower rates. The higher rate treatments (those above 1/10 of the targeted full rate) were not much different than those treated at the full rate (−34 % change in NDVI versus −24 % change), but were different from the untreated containers (2 % change in NDVI). The greater decrease in NDVI with the fractional rates versus the full rate treatment was likely due to better plant coverage with the smaller droplet spray. Deposition and percentage change in NDVI were negatively correlated (PCC=−0.89, $P<0.0001$, and $n=60$). The best fit model was an exponential association [Eq 4 and Fig. 10] with a standard error of 8.8 and a correlation coefficient of 0.90. Additionally, the percent change in NDVI was strongly positively correlated with the percent change in height (PCC=0.72, $P<0.0001$, and $n=60$)

$$\text{exponential association: } y = a(1 - e^{-bx}) \tag{4}$$

where:

x=deposition (percent of 46.8-L/ha rate),
y=percent change in NDVI 9 DAT,
a=−82.6, and
b=0.022.

Conclusions

A protocol was established to perform laboratory bioassays of glyphosate spray drift at depositional rates and spray droplet sizes corresponding to values observed in previously performed field trials. Targeted fractional deposition treatment levels were attained using a laboratory spray table through calibration of nozzle traverse speed and position of target under the nozzle. The established treatments were used to apply glyphosate on greenhouse grown bermudagrass samples. Pre- and postspray plant health measures (plant height and NDVI) were obtained. The measured deposition levels and plant health measures were successfully used to develop bioassay curves useful for predicting biological impacts of spray drift. This methodology is one that can be implemented across a wide variety of plant and pesticide combinations. Data collected from ongoing studies of this type will be tested in full scale aerial drift studies and will ultimately be included in application decision support systems that integrate spray drift modeling results with established dose-response relationships.

Acknowledgments

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